# MIGRATION OF THE PROXIMAL RETINAL PIGMENT IN THE CRAYFISH IN RELATION TO OXYGEN DEFICIENCY

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The primary factors determining the position of the pigment in the proximal and distal sets of pigmented cells of the crustacean retina (Fig. 1) are light and darkness. Whether they act directly on the cells or indirectly via the circulation or the nervous system has not been definitely determined. But ever since Exner (1891) called attention to the optical importance of ensheathing the visual axis (Fig. 1, rhabdome and cone) of the ommatidium with opaque pigment in the light and of withdrawing this sheath in darkness, thereby allowing the photoreceptor (the rhabdome) to make use of light other than that which enters along its axis, most investigators have regarded this as a more or less adaptive mechanism, though of somewhat doubtful effectiveness.

It became apparent, however, that under some circumstances factors other than light and darkness might induce changes in the proximal and distal sets of pigmented cells of the crustacean retina. For example, Congdon (1907) found that temperature was such a factor in certain Crustacea. Demoll (1911) and Bennitt (1924) found that anaesthesia and death, in noctuid moths and crustaceans respectively, were always accompanied by the extreme "light-adapted" position of the retinal pigment, regardless of the surroundings. Welsh (1930a) recently noticed that anaesthesia had this effect on the distal retinal pigment of the shrimp *Macrobrachium*. He further observed a "diurnal rhythm" in the migrations of the distal pigment cells in the retina of this animal, even under constant illumination, and this he believed to be associated with the same sort of "metabolic periodicity" to which had been ascribed numerous other cases of periodic change— in color, luminescence, etc. Bennitt (1932b) observed a similar, though less extensive, diurnal rhythm in the proximal pigment cells of the retina in crayfishes which were kept for several days in total darkness. Finally, Bennitt (1924, 1932a) obtained evidence that under certain conditions stimulation of one eye might result in bodily changes affecting the position of the proximal pigment in the other (unstimulated) eye; whether these changes are nervous or vascular or both is still undecided.

It would seem, therefore, that in addition to the usual photic changes associated with the action of the proximal pigmented cells of the retina there is an internal mechanism of some sort whose operation may, under certain conditions, bring about changes which were at first thought to be solely photomechanical. The experience of Demoll, Bennitt, and Welsh with anaesthetics, referred to above; Arey's (1916) discovery that oxygen deficiency was associated with retraction of the
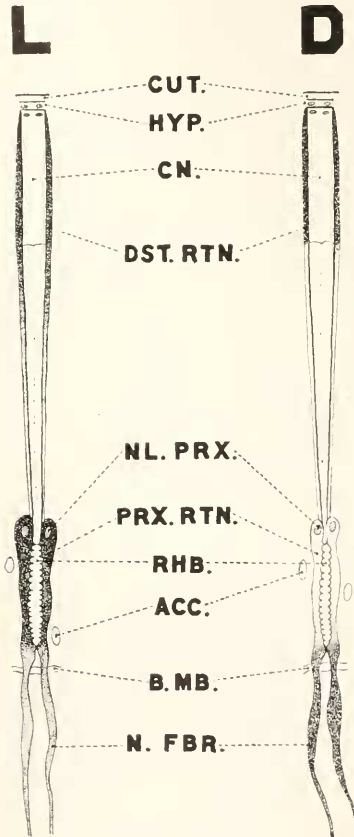


FIG. 1. Longitudinal sections of two ommatidia of *Cambarus virilis* Hagen, showing the arrangement of retinal pigment in the light-adapted (*L*) and dark-adapted (*D*) conditions. Only two of the seven proximal retinal pigment cells are shown in each ommatidium.

*ACC.*, nuclei of accessory pigment cells; *B.MB.*, basement membrane; *CN.*, cone; *CUT.*, cuticle; *DST.RTN.*, distal retinal pigment cells; *HYP.*, cells of the hypodermis; *N.FBR.*, prolongations of the proximal retinal cells which form nerve-fibers into the optic ganglia; *NL.PRX.*, nuclei of proximal retinal pigment cells; *PRX.RTN.*, proximal retinal pigment cells; *RHB.*, rhabdome.

× 125.

retinal pigment in fishes; the work of Spaeth (1913) on the melano-
phores of fishes and of Uyeno (1922) on those of the frog, both of which
showed distinct pigmentary changes accompanying oxygen want; and
some early observations of the writers and others on the effect of
overcrowding crayfish in an aquarium—all these led us to investigate
the specific effect of oxygen deficiency on the action of the retinal
pigment cells of the crayfish. We limited this investigation to
observations on the *proximal* pigment cells, in which pigment streams
distally in the light and proximally in darkness through the cytoplasm
of cells of virtually fixed length (cf. Fig. 1 and Plate 1). Photo-
mechanical movements of the distal cells have been described in the
shrimp *Palaemonetes* by Welsh (1930b).

When a crayfish is "light-adapted" (Fig. 1, *L*; Plate 1, *L*), anaes-
thesia or death evoke no change in the position of the proximal
pigment. When, however, the crayfish is "dark-adapted" (Fig. 1, *D*;
Plate 1, *D*), death, anaesthesia, or simply overcrowding in the aquarium
induce distal migration of the proximal retinal pigment even though
the animal is in total darkness throughout the experiment.

## GENERAL METHODS

The crayfishes, *Cambarus virilis* Hagen and *C. clarkii* Girard, were
killed in hot water to fix the position of the pigment, and were ex-
amined: (1) after sectioning in paraffin, or (2) after macerating for 18
hours or more in Bela Haller's fluid. The latter method was simpler,
and was equally reliable for the study of proximal cells, especially
since the rhabdomes and accessory pigment cells usually adhered to the
dissociated proximal pigment cells.

Oxygen and nitrogen were taken from commercial cylinders.
Carbon dioxide was taken first from a Kipp generator, later from a
commercial cylinder; no difference in results was detected.

Oxygen determinations were made by the Winkler method,
modified after Kemmerer, Bovard, and Boorman (1923). Free carbon
dioxide was determined by the method of Seyler (1894). The pH was
determined colorimetrically by the use of the Clark and Lubs standard
indicators bromthymol blue, bromcresol purple, and cresol red.

The temperature of the water was taken with each sample, and the
variation was found to be within $3°$ C. for any given experiment.

The exact position of the proximal retinal pigment was recorded in
every case, but for convenience in tabulation three general positions
are used in this paper: (1) "Dark"— pigment not distal to the bases of
the rhabdomes (Fig. 1, *D*; Plate 1, *D*); (2) "intermediate"—pigment
extending along the rhabdomes but not distal to them; (3) "light"—
pigment extending beyond the distal ends of the rhabdomes, *i.e.*,

PLATE I
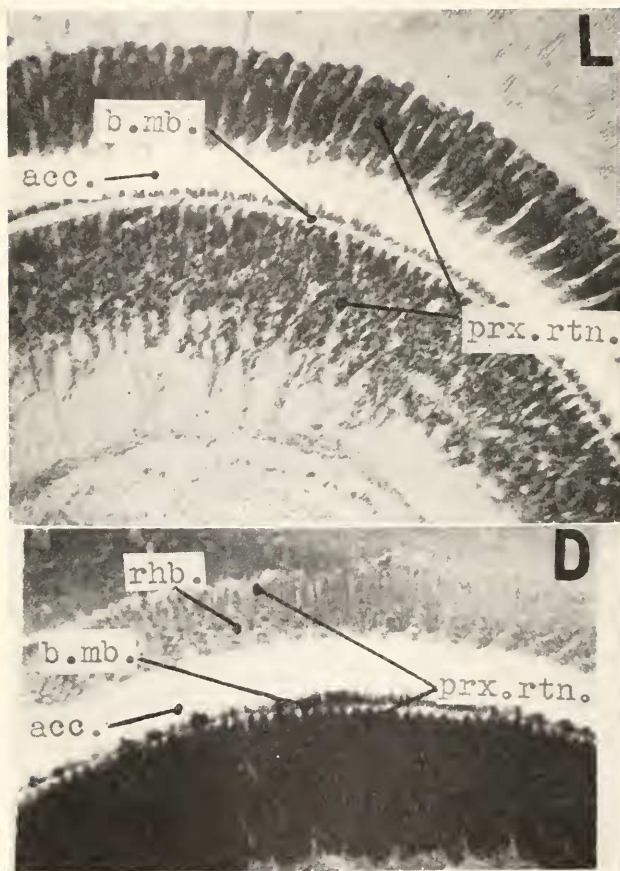


Photographs of unstained sections of the compound eyes of *Cambarus virilis*, in the light-adapted (*L*) and dark-adapted (*D*) conditions. Only a part of each retina is shown, and only the proximal components of the ommatidia (cf. Fig. 1), *viz.*:

*acc.*, accessory pigment layer; *b.mb.*, basement membrane; *prx.rtn.*, proximal retinal pigment cells, showing the nuclei at their distal ends, and extending proximally through the basement membrane; *rhb.*, rhabdomes, visible in (*D*) as the crenulated areas among the proximal retinal cells, but obscured in (*L*) by the retinal pigment. × 125.

nearly or quite to the tips of the proximal pigment cells (Fig. 1, *L*; Plate 1, *L*).

## Experiments

### General Effect of Excess Carbon Dioxide

Preliminary experiments were performed in this laboratory by Mr. L. M. Schmidt.  He did not determine the oxygen content or carbon dioxide content of the water, but he found that exposure to an excess of carbon dioxide (1) inhibited proximal migration of the proximal retinal pigment when light-adapted crayfish were placed in the dark and (2) brought about distal migration of this pigment in dark-adapted animals which were kept in darkness.   His method was

### Table I

*General effect of excess carbon dioxide on the position of the proximal retinal pigment —preliminary experiments.*

|  | Number of eyes examined | | |
|---|---|---|---|
|  | Dark | Intermediate | Light |
| Controls—killed at end of experiment after 6 hours in darkness........................................ | 12 | 2 * | 0 |
| Light-adapted animals, placed in darkness for 6 hours in dishes through which $CO_2$ bubbled at rate of 2 liters per hour....................................... | 0 | 0 | 18 |
| Controls—killed in darkness at beginning of exposure to $CO_2$.......................................... | 7 | 6 * | 0 |
| Dark-adapted animals, kept in darkness for 6 hours thereafter in dishes through which $CO_2$ bubbled at rate of 2 liters per hour......................... | 0 | 0 | 34 |

* The observed variation in pigment-position in the proximal cells of dark-adapted eyes was probably due to the fact that some of the controls were killed during the day, others at night.   Bennitt (1932*b*) found evidence of diurnal variation in pigment position, even when the crayfish were in continuous darkness.

to bubble carbon dioxide (2 liters per hour) for six hours through water containing a number of crayfish, keeping other animals in running water as controls.   All his animals were alive and were moving their swimmerets at the end of the six-hour period.   Schmidt's results appear in Table I.

### Relation between Position of Proximal Retinal Pigment, Carbon Dioxide Content, Oxygen Content, and Hydrogen Ion Concentration

Carbon dioxide was bubbled through about five inches of water in a 12 x 18 inch aquarium at the rate of 3 liters per hour.   Eight or ten dark-adapted crayfish in the aquarium were prevented from rising

to the surface by a layer of sheet cork. The apparatus was kept in darkness, and at intervals of one or two hours animals were killed and water samples were taken for determination of pH, oxygen content, and carbon dioxide content.

The experiments involved 42 animals (84 eyes); 21 animals (the "dark controls") were killed at the beginning of the exposure to carbon dioxide; the other 21 were kept in water through which carbon dioxide passed, and were in darkness throughout. The results appear in Table II, in which the figures for oxygen content, carbon dioxide content, and pH represent averages for the five experiments.

## TABLE II

*Relation between position of the proximal pigment, oxygen content, carbon dioxide content, and pH.* Carbon dioxide bubbled through the water at the rate of 3 liters per hour. Animals in darkness throughout the experiment. (*—cf. foot-note, Table I.)

|  | Oxygen | $CO_2$ | pH | Number of eyes examined | | |
|---|---|---|---|---|---|---|
|  |  |  |  | Dark | Intermediate | Light |
|  | *cc./liter* | *cc./liter* |  |  |  |  |
| Dark controls................ | 3.2 | 1.2 | 7.6 | 34 | 6 * | 2 * |
| Continued in darkness; $CO_2$ for 2 hrs. at rate of 3 liters per hr.. | 0.8 | 45.0 | 6.0 | 0 | 6 | 12 |
| Same conditions—4 hrs........ | 0.0 | 66.0 | 5.3 | 0 | 0 | 12 |
| Same conditions—6 hrs........ | 0.0 | 124.0 | 5.3 | 0 | 0 | 12 |

## TABLE III

*Relation between position of the proximal pigment, oxygen content, carbon dioxide content, and pH.* Carbon dioxide bubbled through the water at the rate of 6 liters per hour. Animals in darkness. (*—cf. foot-note, Table I.)

|  | Oxygen | $CO_2$ | pH | Number of eyes examined | | |
|---|---|---|---|---|---|---|
|  |  |  |  | Dark | Intermediate | Light |
|  | *cc./liter* | *cc./liter* |  |  |  |  |
| Dark controls................ | 3.4 | 35.4 | 7.7 | 2 | 4 * | 0 |
| Continued in darkness; $CO_2$ for 30 min. at rate of 6 liters per hr............................ | 0.2 | 137.5 | 5.4 | 2 | 4 | 0 |
| Same conditions—60 min...... | (water-sample lost) | | | 4 | 2 | 0 |
| Same conditions—90 min...... | 0.0 | 277.1 | 4.4 | 0 | 0 | 6 |

As the experiment progressed, the carbon dioxide content in each case increased while the pH and the oxygen content decreased. In an attempt to determine whether or not there was any quantitative

relation between the rapidity of pigment migration and the rapidity of change of these physical values, carbon dioxide was next bubbled through the water at twice the former rate (*i.e.*, 6 liters per hour), and animals and water samples were removed every thirty minutes. The results appear in Table III.

All the animals in this experiment were light-adapted at the end of one and one-half hours. When carbon dioxide had been administered only half as rapidly (*i.e.*, 3 liters per hour), this condition was not attained until between three and four hours. The rapidity of migration of the proximal pigment appeared to vary in general with the rapidity of administration of carbon dioxide, though whether or not carbon dioxide itself was the active agent in the migration remained to be seen.

## TABLE IV

*Relation between position of the proximal pigment, oxygen content, carbon dioxide content, and pH.* Nitrogen bubbled through the water at the rate of 2–3 liters per hour. Animals in darkness. (*—cf. footnote, Table I.)

| | Oxygen | $CO_2$ | pH | Number of eyes examined | | |
| --- | --- | --- | --- | --- | --- | --- |
| | | | | Dark | Intermediate | Light |
| | *cc./liter* | *cc./liter* | | | | |
| Dark controls (beginning) . . . . . | 2.1 | 2.7 | 7.5 | 20 | 6 * | 10 * |
| Continued in darkness; $N_2$ for 2 hrs. at rate of 2–3 liters per hr. | 0.6 | 8.0 | 7.4 | 0 | 8 | 20 |
| Same conditions—4 hrs. . . . . . . . | 0.3 | 7.7 | 7.4 | 2 | 2 | 26 |
| Same conditions—6 hrs. . . . . . . . | 0.0 | 14.2 | 7.3 | 0 | 0 | 12 |
| Dark controls (end) . . . . . . . . . . | — | — | — | 18 | 12 * | 6 * |

Comparing now the physical conditions, shown in Tables II and III, which obtained when distal migration was complete in all the eyes, it appeared that while the oxygen content dropped to zero in both cases, there was little uniformity in the values for either carbon dioxide content or pH. The next step was to determine whether the pigmentary response was associated with (1) surplus carbon dioxide, (2) oxygen deficiency, or (3) increase in hydrogen ion concentration.

### *Effect of Oxygen Deficiency with Only a Slight Increase in Carbon Dioxide Content*

Instead of carbon dioxide, nitrogen was bubbled through the water at the rate of 2 to 3 liters per hour. This permitted the free oxygen to be used up as in the preceding experiments without at the same time adding artificially a large amount of carbon dioxide to that already present. The procedure was as before except that "dark controls,"

kept in running water, were killed at both the beginning and the end of each exposure to nitrogen. The results of the six experiments are summarized in Table IV.

Since complete distal migration occurred in all animals killed when the $CO_2$-content was as low as 14.2 cc. per liter in the experiments with nitrogen, while it did not occur at much higher concentrations of carbon dioxide in previous experiments (cf. Tables II and III), it appeared that the concentration of carbon dioxide as such was not a factor affecting pigment migration in the proximal retinal cells except insofar as it might contribute to other physical changes. Moreover, since distal migration occurred in all cases at a pH as high as 7.3 in the

### TABLE V

*Relation between position of the proximal pigment, oxygen content, carbon dioxide content, and pH. Water acidified with HCl. The pH-range represents readings taken at the beginning and end of each experiment.*

| | Oxygen | $CO_2$ | pH range | Number of eyes examined | | |
|---|---|---|---|---|---|---|
| | | | | Dark | Intermediate | Light |
| | *cc./liter* | *cc./liter* | | | | |
| Dark controls. . . . . . . . . . . . . | 1.7 | 1.5 | 7.6–7.6 | 12 | 0 | 0 |
| Continued in darkness; acidified water for 2 hours. . . . . | (no determinations) | | 5.2–5.6 | 4 | 2 | 0 |
| Same conditions—4 hours. . . | 0.2 | 5.6 | 5.0–6.0 | 8 | 4 | 2 |
| Same conditions—2 hours. . . | 3.4 | 44.0 | 3.9–4.8 | 2 | 8 | 16 |

experiments with nitrogen, while failing to occur at a pH as low as 6.0 in the earlier experiments (cf. Tables II and III), it would seem that the hydrogen ion concentration also was not a directly contributing factor. However, additional experiments were undertaken to settle this point.

### *Effect of Altered Hydrogen Ion Concentration*

Tap-water was acidified with HCl, and dark-adapted animals were left in it in darkness for varying periods. The "dark controls," as before, were kept in running water. The results of these experiments are listed in Table V.

Thus hydrogen ion concentration appeared to become an active factor in promoting distal migration of the proximal retinal pigment only at acidities as high as pH 3–4, and even under these conditions the effect was by no means consistent, as is indicated by the 10 eyes which failed to become light-adapted. With one exception, however, the pH of the water in all previous experiments did not go below 5, and in all

these cases distal migration had proceeded to completion. Hydrogen ion concentration, within the experimental range produced by the addition of carbon dioxide, did not affect appreciably the position of the pigment.

## DISCUSSION

It should be noted that the animals were neither dead nor even completely quiescent at the end of maximum exposures to carbon dioxide, nitrogen, or acidified water. In all cases the swimmerets were moving and in many cases the mouth-parts and other appendages as well. Check experiments showed that these animals could stand the most unfavorable conditions to which they were subjected in the experiments described in this paper, and would recover when replaced in running water.

The only one of the three factors tested which showed any consistent relation to migration of the proximal retinal pigment was oxygen content. As this decreased, the pigment began to move toward the distal position, even in darkness, and this migration was invariably completed when the oxygen tension had dropped to zero. If we are correct in supposing that in aquatic Crustacea there is a fair degree of correlation between the oxygen content of the water and the metabolic activity of the animal (as expressed by its oxygen consumption) it seems reasonable to believe that the pigmentary changes here observed are associated in some way with varying metabolic conditions. Evidence that the supposition is correct is afforded by Amberson, Mayerson, and Scott (1925). In a large number of experiments on the lobster (*Homarus*) they found a correlation between oxygen tension in the water and oxygen consumption by the animal so close as to warrant the statement that "at every instant the oxygen-consumption is directly proportional to the oxygen-tension in the sea-water at that instant" (1925, p. 175). They found an equally close correlation of the same sort in the polychaet *Nereis*, and preliminary experiments indicated a similar condition in the horse-shoe crab (*Limulus*), the blue crab (*Callinectes*), and the shrimp (*Palaemonetes*). *Palaemonetes* was able to keep its oxygen consumption constant until the oxygen content of the surrounding water had dropped to 50 per cent of saturation, but when less oxygen than this was present, its response was similar to that of the lobster.

Since distal migration of the proximal retinal pigment, though influenced primarily by the presence of light, is promoted also by low temperature, oxygen deficiency, anaesthesia, and death, it begins to look as though it might be promoted by any general factor which tends to retard metabolic activity. This idea was first presented by W. H.